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Letter to the Editor

Spontaneous and Isoprenaline-evoked response of isolated heart preparations from rats submitted to leptin treatment during lactation

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Stressful events during gestation and lactation have a strong correlation to the development of adult diseases such as obesity and cardiovascular failure [1]. High serum concentrations of leptin in the first 10 days of lactation were associated with hyperleptinemia at 150 days of age and also with a higher food intake and body weight [2]. These data reflect a resistance to the anorectic effect of leptin, as observed for animals whose mothers were exposed to malnutrition during lactation [2,3].

Trevenzoli et al. had previously described that leptin treatment during the first 10 days of lactation increased catecholamine synthesis and secretion [4]. Echocardiographic studies have shown cardiac dysfunction compatible with diastolic heart failure in this experimental model in early age that evolved to systolic dysfunction in adulthood [5]. The aim of the present work was to study the spontaneous cardiac activity of isolated heart preparations from rats at 30 and 150 days of age treated with leptin during the first 10 days of lactation, also investigating their response to β-adrenergic stimulation.

This study was performed at the Laboratory of Experimental Pharmacology of Fluminense Federal University and approved by local Ethics Committee (CEPA/UFF00123-09). Wistar rats were treated with leptin (PeproTech Inc., London, UK) at a dose of 8 mg/100 g body weight daily for the first 10 days of lactation or saline (Control-Leptin naïve) as previously described [2,4,5].

Rats were heparinized before being sacrificed by carbon dioxide inhalation and cervical dislocation. The hearts were rapidly excised following a mid-line thoracotomy, and the aorta was cannulated using a Langendorff apparatus, perfused retrogradely at constant flow with Krebs–Henseleit modified buffer (in mmol/L: 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.25 $CaCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 11 glucose), and equilibrated with 95% O_2/5% CO_2 gas mixture at 37.0 ± 0.5 °C. A water-filled latex balloon was placed into the left ventricle (LV) through the mitral valve and connected to a pressure transducer and PowerLab System (ADInstruments, Australia) for continuous LV pressure recording. The heart was kept immersed in a buffer-filled water jacketed glass chamber, and the end-diastolic pressure (LVEDP) was adjusted to 10 mm Hg. LV developed pressure (LVDP) was determined as the difference between the peak systolic and LVEDP [6]. Cardiac performance indexes such as LV contractility rate (assessed by + dp/dt) and LV relaxation rate (assessed by − dp/dt) were also determined. After stabilization and basal parameters recording, the hearts were subjected to increasing concentrations of isoprenaline (1, 10 and 100nM), a non-selective β-agonist [7].

All data are expressed as mean ± SEM. A two-tailed unpaired Student's t-test was performed and statistical significance was accepted at the 0.05 level (Graph-Pad Prism 5.0).

Fig. 1 shows spontaneous response of isolated heart preparations from rats at 30 and 150 days of age. Analyzing the left ventricle relaxation rate it is possible to observe that the hearts of rats at 30 days of age submitted to neonatal leptin treatment presented a lower spontaneous lusitropic activity when compared to control, without changes of spontaneous inotropic activity. These data corroborates the diastolic dysfunction suggested by Marques et al. in this experimental model [5]. Heart preparations from rats at 150 days of age of Leptin group presented a lower spontaneous inotropic activity when compared to control, considering both LV developed pressure as the systolic contractility rate (Fig. 1). These data also corroborate the findings of Marques et al. whose echocardiographic studies have inferred a systolic dysfunction in these older animals [5].

Given the important role of sympathetic activity in the pathophysiology of heart failure [8], the concentration-dependent response to isoprenaline of perfused hearts was also evaluated (Table 1). Preparations from rats of Leptin group at 30 days of age showed a greater increase of LV developed pressure, as well as of both cardiac performance indexes, with 100 nM isoprenaline. On the other hand, it was just observed a

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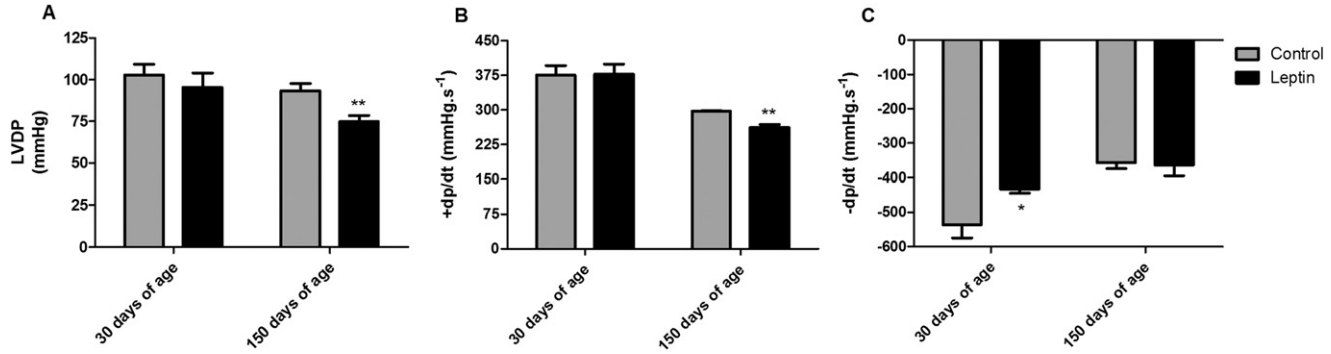

Fig. 1. Spontaneous response of heart preparations from rats that receive saline (Control-Leptin naive-gray bars) or leptin (Leptin-black bars) during lactation at 30 (n = 4) and 150 (n = 4-7) days of age. The analyzed parameters were (A) left ventricular developed pressure, (B) left ventricular contractility rate, (C) left ventricular relaxation rate. Values are mean ± SEM. *P < 0.05 or ** P < 0.001-Leptin vs Control.

greater increase of LV contractility related to neonatal leptin treatment in hearts from older animals. These data are also in accordance to Marques et al. whose electrocardiographic studies suggested the raise of sympathetic response [5].

Physiological sympathetic stimulation of the heart through β-adrenergic receptors is related to developed contraction rise, relaxation acceleration and intracellular calcium concentration decline [9]. Stimulation of β-adrenergic receptor activates a GTP-binding (G) protein, which stimulates adenylyl cyclase to produce cAMP, which in turn activates PKA. This kinase phosphorylates several proteins related to excitation–contraction coupling [9].

Heart failure is characterized by a complex interplay of several neurohormonal mechanisms that get activated in the syndrome in order to sustain cardiac output in the face of decompensating function. Perhaps the most prominent among these mechanisms is the sympathetic nervous system. Acutely this rise of the adrenergic activity will promptly restore cardiac function [8]. In offspring of obese dams, systolic and diastolic dysfunction was associated with cardiac sympathetic dominance that can be explained by upregulation of β1 adrenergic receptors [7]. So, as Marques et al. described a diastolic dysfunction with preserved ejection fraction in rats at 30 days of age submitted to neonatal leptin treatment that evolves to systolic dysfunction in rats at 150 days of age [5] and as the data from Langendorff assay is obtained using denervated hearts, we postulate that the greater response to isoprenaline of heart preparations from the younger Leptin group may be due to the rise of β1-adrenergic receptor expression and/or of efficacy of its intracellular signaling machinery. However, hyperstimulation or overexpression of β1-adrenergic receptor has detrimental effects in the heart [8]. In systolic heart failure cardiac β-adrenergic dysfunction is generally characterized at the molecular level by selective β1-adrenergic receptor downregulation and by uncoupling of the remaining receptors from G proteins [8], which may explain the absence of a greater effect of isoprenaline on LV developed pressure and on LV relaxation rate in the older Leptin group. As the ability of sympathetic nervous system to maintain cardiac function decreases in the course of chronic heart failure, sympathomimetics used as positive inotropes for acute heart failure significantly increase mortality if used chronically [8]. Several major trials have established the cardiovascular benefits of carvedilol, a β-blocker, in patients with heart failure and this drug is currently used in the pharmacotherapy of heart failure with systolic dysfunction [10].

Thus, our data suggest that spontaneous and sympathetic response of isolated heart preparations from rats submitted to neonatal leptin treatment are compatible with the diastolic heart failure in youth that evolves to systolic dysfunction in adulthood previously described [5]. Studies are in course to precisely delineate the molecular and biochemical mechanisms underlying these changes in order to discuss new pharmacological approaches for heart failure.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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Table 1
Effect of isoprenaline on the spontaneous response of heart preparations from rats at 30 and 150 days of age that received saline (Control-leptin naive) or leptin (Leptin) during lactation.

Variables	30 days of age		150 days of age	
	Control n = 4	Leptin n = 4	Control n = 4	Leptin n = 7
LVDP (% basal)				
1 nM	16.8 ± 3.5	26.7 ± 2.6	10.5 ± 2.8	13.6 ± 3.0
10 nM	42.4 ± 9.8	61.7 ± 12.3	56.0 ± 16.8	56.5 ± 8.0
100 nM	70.9 ± 6.5	129.6 ± 22.0*	97.8 ± 4.7	91.2 ± 16.1
+dp/dt (% basal)				
1 nM	463.4 ± 10.1	466.1 ± 49.7	320.9 ± 14.6	316.6 ± 13.2
10 nM	650.7 ± 66.4	869.6 ± 52.7	345.7 ± 35.4	397.8 ± 19.2
100 nM	847.6 ± 21.5	1034.4 ± 24.9**	360.9 ± 44.7	556.5 ± 47.0*
−dp/dt (% basal)				
1 nM	−617.0 ± 18.8	−673.4 ± 15.0	−420.9 ± 17.0	−395.2 ± 17.6
10 nM	−861.5 ± 73.3	−1109.8 ± 65.5	−443.7 ± 51.0	−505.6 ± 33.0
100 nM	−1054.1 ± 37.1	−1330.3 ± 27.1**	−540.3 ± 86.5	−681.1 ± 55.5

LVDP — left ventricular developed pressure; +dp/dt — left ventricular contractility rate; −dp/dt — left ventricular relaxation rate. Values are mean ± SEM.
*P < 0.05 or ** P < 0.001 — Leptin vs Control.

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